The Effect of Green Coffee Bean Extract on Memory Impairment Induced by Scopolamine in Rats

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ABSTRACT

This study aimed to evaluate the effect of Green Coffee Bean Extract (GCBE) on memory impairment induced by scopolamine in rats. Forty rats were separated into two groups. 1st group, rats (n=8) was fed on basal diet and kept as negative control group, 2st group: (scopolamine group), rats (n=32), were injected with scopolamine (1 mg/kg dissolved in saline, i.p.) for 20 day to induce memory impairment in rats, After ensure the induction of memory impairment then rats were divided into 4 subgroups as follow: subgroup 1 served as the control positive group and 3 treated rat subgroups were orally given 1ml/day of green coffee bean extract at concentration of (5%, 7.5% and 10%), respectively. The results revealed that scopolamine induced promoted acetyl cholinesterase activity, β-amyloid levels and oxidative stress. On the other hand, GCBE demonstrate a significant reduced Acetylcholine esterase activity, β-amyloid levels and oxidative stress markers (MDA) which, improves memory, and antioxidant enzyme levels (SOD, CAT and GSH) in scopolamine-induced rats. Moreover, GCBE to ameliorate the neurological (norepinephrine, dopamine and serotonin), lipid profile (TC, TG, LDL, HDL and VLDL) and liver (ALT, AST and ALP), kidney function (Urea, Creatinine and Uric Acid) in rats. Accordingly, the present study demonstrates the beneficial effects of green coffee bean extract against scopolamine-induced memory impairment. Conclusion, GCBE might become a promising agent for memory improvement in Alzheimer's disease and AD-like disorder.

Keywords: Green Coffee Bean Water Extract, Scopolamine, Alzheimer's disease; Anticholinesterase activity; β-amyloid, Oxidative stress.

INTRODUCTION

Alzheimer's disease (AD) is an irreversible, progressive neurodegenerative illness first defined by a German psychiatrist Alois Alzheimer in 1906 characterized by impairment of short & long memory, abnormal behavior, disorientation, deterioration of motor skills, sleeping disturbance, & impairment of cognitive functions leading to disability to carry on simple daily tasks followed by total dependence on caregivers. AD is considered the sixth leading cause of death **(Kumar et al., 2022)**, with approximately 6.5 million afflictions in the USA in 2022 **(Alzheimer's Association, 2022)** & 50 million worldwide **(Breijyeh and Karaman, 2020)**.

Many theories have been implicated in the progression of AD, including Scopolamine (SCOP) is a non-selective post-synaptic muscarinic antagonist that is capable of inducing memory impairment by disturbing cholinergic neurotransmission. Therefore, SCOP could mimic in part the neurodegeneration and memory impairment accompanying AD in rats **(Aykac et al., 2022)**. It has been reported that natural products or plant components, some of which are currently in use as potent AChE inhibitors, can reverse memory deficits in studies using various animal models, including SCOP induced models **(Mohamed et al., 2023)**.

Coffee is one of the most common beverages in the world **(Samoggia & Riedel, 2019)**. Recent studies showed that when consumed in moderation, this soft drink could have beneficial effects on human health thanks to its biological properties **(Carneiro et al., 2021)**. Green coffee in particular, is the bean of the Coffee fruit that has not yet been roasted, meaning it is able to maintain more bioactive phytochemicals than common roasted coffee beans. Further, green coffee beans are rich in a polyphenol called chlorogenic acid (CGA), prepared

from unroasted and decaffeinated coffee beans to avoid the probable side effects of caffeine **(Song et al., 2014)**.

CGA is a natural chemical compound which is the ester of caffeic acid and quinic acid. Both caffeinated and decaffeinated coffee contain chlorogenic acids such as caffeoylquinic acids, feruloylquinic acids, dicaffeoylquinic acids, and pcoumaroylquinic acids, in smaller amounts **(Kim et al., 2012)**. In addition to caffeine, green coffee contains various isomers of CGAs which are strong free radical scavengers **(Priftis et al., 2018)**. The extract of Coffee Arabica beans has also been reported to exert neuroprotective effects by reducing cognitive and memory impairment **(Mohamed et al., 2021; Carneiro et al., 2021)** as antioxidants, or by preventing amyloid formation and neurotoxicity **(Ciaramelli et al., 2019**). Therefore, this study was conducted to investigate the effect of green coffee bean extract on memory impairment induced by scopolamine in rats.

MATERIALS AND METHODS

MATERIALS:

1- **Green Coffee beans:** were purchased from Agriculture Research Centre.

2- Chemicals: Casein, cellulose, choline chloride, D-L methionine, vitamins, minerals, and Scopolamine constituents were purchased from El Gomhoriya Pharmaceutical Company, Cairo, Egypt. Starch, soybean oil and sucrose were obtained from the local market.

3- Kits: for blood analysis were purchased from Alkan Company for Biodiagnostic Reagents, Dokki, Cairo, Egypt.

4- Animals: 40 adult male rats (Sprague Dawley strain) were obtained from National Research Center, Dokki, Egypt.

METHODS:

Experimental study was conducted according to the guidelines of Animal Care and Ethics Committee of the NRC as well as the biochemical analysis at the Postgraduate Lab of the Faculty of Home Economics, Helwan University.

4.2.1 Preparation of Green Coffee beans:

The green coffee beans were grinded into fine powder using an electrical blender. The obtained powder was soaked in water (70 °C) for 20 min like the traditional way to prepare a coffee drink by Arabians. After that, the extract was filtered and lyophilized to remove water. The final physical appearance of the extract will be powder. The extract was defined as a GCBWE (green coffee bean water extract), dissolved in water to be 100 mg/ml and stored at −20 °C until use.

4.2.2 Experimental Design

The experimental animal were done using (n=40) male rats, with body weight (180 ±10) g. The rats were housed in cages under hygienic conditions, at temperature-controlled room 25°C. Basal diet was semi-synthetic and nutritionally adequate (AIN-93 M), vitamins mixture and minerals mixture were prepared as described by **Reeves *et al*., (1993).** After 5 days, rats were randomly divided into two main groups as follows:

The first main group (n= 8): animals were served as the negative control group, which received only basal diet.

The second main group (N=32): (scopolamine group) that received scopolamine (1 mg/kg dissolved in saline, i.p.) injection for 20 day to induce memory impairment in rats **(Ghasemi *et al*. 2019)**, To ensure the induction of Alzheimer Disease, blood samples were taken from 4 rats to determine amyloid beta concentration in serum.

Then rats were divided into 4 subgroups as follows:

Subgroup (1): Rats (served as positive control group) were fed on basal diet only

Subgroup (2): received scopolamine (1 mg/kg) and orally given 1ml/day of Green Coffee extract at concentration of 5%.

Subgroup (3): received scopolamine (1 mg/kg) and orally given 1 ml/day of Green Coffee extract at concentration of 7.5%.

Subgroup (4): received scopolamine (1 mg/kg) and orally given 1 ml/day of Green Coffee extract at concentration of 10%.

4.2.3 Nutritional evaluation:

The biological evaluation of the diet were carried by determination of feed intake, body weight gain percent (BWG %) and feed efficiency ratio (FER) according to **Chapman, (1959)**.

$$\textbf{BWG \%} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

FER = Weight gain (g) / Feed intake (g).

At the end of the experimental period (30 days), rats were fasted overnight, then the blood were collected under slight ether anesthesia. Serum was

separated by centrifugation at 3000 rpm for 15 min. The obtained serum were used immediately for routine laboratory investigation.

Biochemical Analysis:

Brain neurotransmitters

Determination of norepinephrine, dopamine (DA) and serotonin (5-HT) were carried out as reported by **(Hussein *et al.*, 2016)** utilizing high performance liquid chromatography (HPLC) system. The Acetylcholine esterase (AChE) and Amyloid beta concentration in brain were assessed in accordance to **(Ellman *et al.*, 1961)**.

Serum Lipid Profile:

Serum total cholesterol (TC) **(Allain,1974)**, triglycerides (TG) (**Fassati and Prencipe,1982)**, High density lipoprotein (HDL) **(Albers *et al.*, 1983)** were determined. Meanwhile, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) was calculated according to **Fridewald *et al.*, (1972)**.

LDL-c = TC-[HDL-c + (TG/5)] VLDL-c = TG/5

Liver Function:

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to (**Bergmeyer *et al.*, 1978)**, Alkaline phosphates (ALP) was determined according to **Belfield and Goldberg (1971).**

Kidney function:

Serum urea **(Kaplan, 1984)**, uric acid **(Patton and Crouch, 1977)** and creatinine were measured according to **(Murray, 1984)**.

Oxidative and Antioxidant Biomarkers:

Following **Draper and Hadley (1990)** methodology, the plasma level of malondialdehyde (MDA) was calculated to measure lipid peroxidation. Superoxide dismutase (SOD) activity was evaluated by **Spitz and Oberley, (1989).** Catalase (CAT) was measured by (**Aebi, 1984)**.

Statistical analysis:

All data were be expressed as mean ± standard error of the mean (SEM). Distribution of the data were be verified to be normal using tests of normality (SPSS version 25). Statistical significance was be tested by one-way analysis of variance (ANOVA). The probability of ($p < 0.05$) was be considered statistically significant **Snedecor and Cochran, (1989)**.

RESULTS AND DISCUSSION

Results in **Table (1)** the initial body weight showed no significant differences among the groups. The scopolamine-injected group showed had significant ($p<0.05$) decrease FBW, FI; BWG% and FER in scopolamine group as compared to the –ve group. In addition, brain weight of the negative group was 0.50 g, while that of the scopolamine treated group decreased to 0.43 g. The reduction of the whole brain weight is usually observed in AD patients **(Burns et al., 2010)**. According to **Li et al., (2018)**, the scopolamine induced memory impairment mice had a decreased ratio of the brain weight to body weight. **Kim et al., (2019**) showed that the whole brain weight decreased in the scopolamine-treated group, suggesting that the scopolamine-treated memory impairment may result in brain atrophy in mice.

However, the administration of green coffee bean extract (GCE) 1ml levels at (5, 7.5 and 10%) increased in FBW, FI; BWG%, FER and brain weight

compared to the positive control group (+ve group). Rat's treatment with green coffee bean extract 1ml levels at (10%) recorded the best result in enhancement (FBW, FI BWG% and brain weight). The obtained results agree with **Kanchanasurakit et al., (2023)** showed that green bean coffee extract (GBCE) supplements reduced body weight. **Elpasty et al., (2022)** denoted that there was a significant reduction in % change of the BW in rats administered GCE relative to the untreated obese rats after 2 and 4 weeks. **Hussein et al., (2020)** who demonstrated that treatment with GCE in high-fat diet (HFD) induced obese rats significantly reduced BW than the untreated group. Another study showed that the administration of GCBE resulted in weight loss in these obese rats **(Salamat et al., 2018)**. Also, the weight reduction effects of GCBE may be attributed to the phytochemical compounds, especially chlorogenic acids (CGAs), caffeine, and other polyphenolic compounds in (GCBE) act synergistically to suppress body weight gain in rats **(Meng et al., 2016)**.

Table (1): The effects of green coffee bean Water extract on Initial Body Weight (IBW), Final Body Weight (FBW), Feed Intake (FI), Body Weight Gain (BWG), Feed Efficiency Ratio (FER) and brain weight of memory impairment rats

Parameters / Groups	IBW G	FBW g	FI (g/d/rat)	BWG %	FER	Brain Weight
Control (-Ve)	183.8±0.71a	225.0±1.23a	**22**	22.41±0.37a	0.0446±0.001a	0.50±0.04a
Control (+Ve) (scopolamine)	185.4±0.81a	207.8±1.86d	**15**	12.08±0.28d	0.0355±0.001b	0.43±0.02d
1ml GC 5%	186.8±0.86a	212.0±1.01c	**17**	13.49±0.19d	0.0352±0.001b	0.45±0.01c
1ml GC 7.5%	187.0±0.70a	220.8±1.66b	**18**	18.07±0.35c	0.0446±0.001a	0.47±0.03b
1ml GC 10%	186.6±0.76a	223.4±1.07ab	**20**	19.72±0.24b	0.0438±0.001a	0.49±0.02a

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at ($P<0.05$).

Results in **Table (2)** showed that the effects of green coffee bean extract on norepinephrine, dopamine and serotonin of memory impairment rats. Injection with scopolamine caused to significant ($P<0.05$) decreased mean value of norepinephrine, dopamine and serotonin in the scopolamine group as compared to the negative control group. Scopolamine a non-selective muscarinic blocker that blocks cholinergic transmission and causes impaired memory loss in mice **(Konar et al., 2019)**. Scopolamine models used are considered most consistent and devour a value in experiments where an AD-like condition is observed **(Tang, 2019)**.

On the other hand, rats that treated with 1 ml of green coffee bean extract at levels (5, 7.5 and 10%) increased in norepinephrine, dopamine and serotonin compared to the positive control group (+ve group). Rats that administration on 1 ml of green coffee bean extract at levels (10%) recorded the highest increasing in norepinephrine, dopamine and serotonin. The obtained results agree with **Al-Brakati et al., (2020)** showed that low dose and high dose of orally administered green coffee bean water extract (GCBWE) co-administration caused improved levels of cortical dopamine, norepinephrine and serotonin with marked increases in their metabolites. Also, **Molska et al., (2021)** showed that GCBWE improved treatment HFD/STZ-induced declines in the neurotransmitters alterations and this is in agreement with previous authors who found that caffeine administration augmented the release of serotonin and dopamine in the hippocampus region **(Owolabi et al., 2017)**.

Table (2): The effects of green coffee bean Water extract on Norepinephrine, dopamine and serotonin of memory impairment rats

Parameters / Groups	Norepinephrine (pg/mL)	Dopamine (pg/mL)	Serotonin (ng/mL)
Control (-Ve)	117.79±0.71a	89.95±0.51a	211.54±0.73a
Control (+Ve) (scopolamine)	64.49±0.69e	70.36±0.65d	173.39±0.88d
1ml GC 5%	72.12±0.48d	72.84±0.55c	176.82±0.93cd
1ml GC 7.5%	75.19±0.44c	75.16±0.26c	180.32±0.86c
1ml GC 10%	85.91±0.49b	81.14±0.47b	193.28±0.64b

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at ($P<0.05$).

Results in **Table (3)** showed that the effects of green coffee bean extract on Acetylcholine esterase and Amyloid-beta of memory impairment rats. Injection with scopolamine caused to significantly increase the expression of Acetylcholine esterase (AChE) and Amyloid-beta in the scopolamine group as compared to the negative control group. The obtained results agree with **Kim et al., (2019)**, injection of scopolamine significantly increased AChE activity in the brain tissue. These results are in line with those of the previous studies **(Brinza et al., 2021; Hosseini et al., 2022)**.

Furthermore, cholinergic transmission plays an important role in the memory and cognitive function. In the cholinergic dysfunction of AD, the AChE enzyme is responsible for the degradation of ACh to acetate and choline and reduces neurotransmitters in the brain **(Dunant and Gisiger, 2017)**. Therefore, the inhibition of AChE can serve as a strategy for the treatment of different forms of dementia, including AD. Additionally, the chronic administration of scopolamine increases Aβ levels at rat hippocampus. It has been widely demonstrated that an increase in the Aβ levels induces oxidative

stress by several mechanisms **(Hernández-Rodríguez et al., 2020)**. However, the expression of AChE and Amyloid-beta in the administration of the green coffee bean extract at levels (5, 7.5 and 10%) significantly decreased compared to the scopolamine-treated group. Rats that administration on 1 ml of green coffee bean extract at levels (10%) recorded the highest inhibit in Acetylcholine and Amyloid-beta. The obtained results agree with **Dhakal et al., (2019)** reported that GCBE inhibits acetyl cholinesterase and butyrylcholinesterase, which would increase acetylcholine in the brain.

Caffeine may be the most likely coffee bioactive responsible to inhibit acetyl cholinesterase, while the inhibitory effects of GCBE on butyrylcholinesterase were associated with its phenolics acids **(Budryn et al., 2018)**. Previously it was reported that GCBE reduced amyloid-β proteins (Aβ) accumulation in the brain of mice used as Alzheimer's disease model **(Ishida et al., 2020)**. Thus, CGA can improve cognitive dysfunction through several pathways and may exert a therapeutic effect in AD.

Table (3): The effects of green coffee bean Water extract on Acetylcholine esterase and Amyloid-beta of memory impairment rats

Parameters / Groups	Acetyl cholinesterase (AChE) (IU/mg protein)	Amyloid-beta Aß42 (pg/mL)
Control (-Ve)	0.74±0.01e	94.0±0.63d
Control (+Ve) (scopolamine)	1.46±0.01a	158.8±1.3a
1ml GC 5%	1.24±0.2b	115.2±0.89b
1ml GC 7.5%	1.04±0.02c	105.57±0.67c
1ml GC 10%	0.95±0.03d	102.62±0.85c

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at (P<0.05).

Results in **Table (4)** showed that the effects of green coffee bean extract on lipid profile of memory impairment rats. Mean value of lipid profile (TC, TG, LDL-C & VLDL-C) significantly ($P<0.05$) increased, while (HDL-c) decreased in the scopolamine group compared with the negative control group. On the other hand, rats that treated with 1 ml of green coffee bean extract at levels (5, 7.5 and 10%) decreased in (TC, TG, LDL-c & VLDL-c) while increased in (HDL-c) compared to the positive control group (+ve group). The best outcomes of improve lipid profile had been found in groups treated with oral administration of 1 ml/day at levels (10%) of green coffee bean extract.

The obtained results agree with **Elpasty et al., (2022)** denoted that administration of GCE induced significant hypolipidemic effects. This result was in agreement with that of **El Rabey et al., (2021)**; **Abdelaal et al., (2019)** showed that the green coffee methanolic extract significantly increased HDL levels and decreased levels of cholesterol, TG and LDL. Also, **Hussein et al., (2020)** who reported that ingestion GCE ameliorated the HFD-caused hyperlipidemia in rats. **Xiaoyun et al., (2020)** showed that GCBE supplementation significantly lowers plasma TC, TG, and LDL-C levels while increasing plasma HDL-C levels in the mice fed HFD. The phytocompounds and CGAs in GCE have been proved to significantly improve lipid content and fat accumulation via activation of fat metabolism in the liver **(Meng et al., 2013)**.

Table (4): The effects of green coffee bean Water extract on lipid profile of memory impairment rats

Parameters / Groups	TC mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control (-Ve)	127.92±0.59b	79.01±0.34d	29.74±0.38a	82.37±0.37d	15.80±0.06d
Control (+Ve) (scopolamine)	141.06±0.53a	92.32±0.32a	24.32±0.31c	98.27±0.48a	18.46±0.05a
1ml GC 5%	139.18±0.44a	89.19±0.35b	25.53±0.81c	95.80±0.39ab	17.84±0.07b
1ml GC 7.5%	138.07±0.65a	87.19±0.55b	27.61±0.41b	93.01±0.49b	17.44±0.10b
1ml GC 10%	132.41±0.21b	83.79±0.47c	28.20±0.37ab	78.45±0.32c	16.76±0.09c

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at ($P<0.05$).

Results in **Table (5)** showed that the effects of green coffee bean extract on Liver Function of memory impairment rats. There was a significant increase at ($p<0.05$) in mean value of AST, ALT and ALP in the scopolamine group as compared to the control negative group. On the other hand, rats that treated with 1 ml of green coffee bean extract at levels (5, 7.5 and 10%) decreased in significant decrease ($p<0.05$) in (AST, ALT & ALP) as compared to the positive control group (+ve group). The best outcomes of improve liver function had been found in groups treated with oral administration of 1 ml/day at levels (10%) of green coffee bean extract. The study outcome is in the same line as **Elpasty et al., (2022)** denoted that the levels of liver function biomarkers in GCE group were comparable with the control levels. **Mosallam, (2022); El Rabey et al., (2021)** showed that the administration of the green coffee extract improved serum levels of these liver function parameters. **Venkatakrishna et al., (2021)** who concluded the safety of green coffee bean extract. **Choi et al., (2016)** confirmed that mice fed green coffee bean extract (GCBE) showed significant decreases in liver function in obese mice.

Table (5): The Effects of green coffee bean Water extract on Liver Function of memory impairment rats

Parameters / Groups	AST (µ /L)	ALT (µ /L)	ALP mg/dL
Control (-Ve)	19.93±0.52d	38.22±0.41e	105.58±0.73e
Control (+Ve) (scopolamine)	44.98±0.42a	94.73±0.59a	171.04±0.97a
1ml GC 5%	42.18±0.47b	81.13±0.45b	155.18±0.92b
1ml GC 7.5%	39.78±0.53b	74.22±0.81c	144.98±0.66c
1ml GC 10%	26.38±0.64c	52.73±0.48d	135.38±0.73d

Results are expressed as mean ± SE.
Values in each column which have different letters are significantly different at ($P<0.05$).

Results in **Table (6)** showed that the effects of green coffee bean extract on kidney function of memory impairment rats. There was a significant increase at ($p<0.05$) in mean value of Urea, Creatinine and Uric Acid in the scopolamine group as compared to the control negative group. On the other hand, rats that treated with 1 ml of green coffee bean extract at levels (5, 7.5 and 10%) decreased in significant decrease ($p<0.05$) in Urea, Creatinine and Uric Acid as compared to the positive control group (+ve group). The best outcomes of improve kidney function had been found in groups treated with oral administration of 1 ml/day at levels (10%) of green coffee bean extract. The study outcome is in the same line as **Elpasty et al., (2022)** denoted that the levels of renal function biomarkers in GCE group were comparable with the control levels. **El Rabey et al., (2021)** showed that the administration of the green coffee extract improved serum levels of these kidney function parameters.

Table (6): The Effects of green coffee bean Water extract on kidney function of memory impairment rats

Parameters / Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric Acid (gm/dl)
Control (-Ve)	22.14±0.21e	0.67±0.05e	2.77±0.05d
Control (+Ve) (Scopolamine)	48.75±0.32a	1.63±0.04a	5.47±0.05a
1ml GC 5%	42.74±0.54b	1.47±0.02b	4.77±0.02b
1ml GC 7.5%	39.12±0.42c	1.34±0.05c	4.12±0.03c
1ml GC 10%	27.41±0.67d	0.96±0.02d	3.05±0.02d

Data are expressed as mean ± SE.

Means with different superscript letters in the column are significantly differences at ($P < 0.05$).

As shown in **Table (7)** the effects of green coffee bean extract on oxidative and antioxidant enzymes of memory impairment rats. The level of oxidative stress parameters like MDA significantly (*P<0.05*) increase, while (antioxidant enzymes) such as (SOD, CAT and GSH) was significantly down-regulated in the scopolamine-treated group as compared to negative control group. Notably, scopolamine also triggers oxidative stress by inducing an imbalance in brain oxidative status **(Kim et al., 2019)**. Our data revealed that scopolamine-induced memory loss was accompanied with increase in oxidative stress in the brain tissue. These results are in line with those of the previous studies (**Brinza et al., 2021; Hosseini et al., 2022**). However, the administration of green coffee bean extract at levels (5, 7.5 and 10%) significantly (*P<0.05*) decrease in oxidative stress parameters like MDA while increased in (antioxidant enzymes) such as (SOD, CAT and GSH) compared to the positive control group (+ve group). The best outcomes of improve oxidative and antioxidant enzymes had been found in groups treated with oral administration of 1 ml/day at levels (10%) of green

coffee bean extract. The obtained results agree with **El Rabey et al., (2021)** observed that the consumption of green coffee extract had a protective effect on oxidative stress (MDA) and antioxidant redox system was reflected by increased concentrations of antioxidants (GSH, CAT and SOD) and a decreased MDA that may be ascribed to its richness in caffeine, which is considered as an important component of coffee.

Similar, **Al-Brakati et al., (2020)** found that green Coffee beans administered group showed down-regulation of the MDA, as compared to the scopolamine-treated group. Consistently, GCBE and CGA increased the expression of antioxidant enzymes, such as SOD, catalase (CAT), glutathione peroxidase (GPx), in rodent brain tissues **(Lee et al., 2020; Metwally et al., 2020; Farias-Pereira et al., 2021)**. The antioxidant activity of green coffee constituents makes it able to trap hydroxyl radicals or superoxide anions. The possible reason for the potent activity of these phenolic acids like chlorogenic acid (CGA), CA, and FA are due to its capability to induce upregulation of cytoprotective enzymes **(Salamat et al., 2018)**.

Table (7): The Effects of green coffee bean Water extract on oxidative and antioxidant enzymes of memory impairment rats

Parameters / Groups	MDA ng/ml	SOD U/ml	CAT Pg/mL	GSH µmol/mL
Control (-Ve)	119.23±0.56d	0.78±0.01a	18.57±0.19a	73.00±0.44a
Control (+Ve) (scopolamine)	355.98±1.71a	0.52±0.05d	7.47±0.14d	53.18±0.39d
1ml GC 5%	341.81±0.48b	0.55±0.01cd	8.21±0.25d	56.78±0.50c
1ml GC 7.5%	340.04±0.84b	0.58±0.01c	10.96±0.46c	58.13±0.48c
1ml GC 10%	287.34±0.71c	0.67±0.03b	15.85±0.47b	65.98±0.46b

Data are expressed as mean ± SE.
Means with different superscript letters in the column are significantly differences at ($P < 0.05$).

Conclusion:

The present study demonstrates that green coffee bean extract improves memory, and antioxidant remarks in scopolamine-induced memory impairment in rats. Consumption of green coffee bean extract seems to scopolamine AD-induced cognitive impairment via decreasing Acetylcholine esterase and Aβ levels while increasing norepinephrine, dopamine and serotonin. Besides, these outcomes offer a scientific justification of green coffee bean extract administration, which might slow the progression of AD. Further clinical studies are warranted to confirm current results and to recommend the regular drinking of green coffee bean extract as a protective approach to delay AD progression in vulnerable individuals or in early disease stages.

REFERENCES

Abdelaal S, Mousa HSE, Ahmed SM. (2019). Effect of Silymarin versus Silymarin and green coffee extract on Thioacetamide induced liver injury in adult male albino rats (histological and Immuno histochemical study). *Egy J Histol*. 42(1):133–46.

Aebi, H. (1984). Catalase in vitro. Methods Enzymol., 105**:**121–126.

Albers, N., Benderson, V. and Warnick, G. (1983). Enzymatic determination of high-density lipoprotein cholesterol, Selected Methods. *Clin. Chem*, *10*(5), 91-99.

Al-Brakati, A., Albarakati, A. J. A., Daabo, H. M. A., Baty, R. S., Salem, F. E. H., Habotta, O. A., Elmahallawy, E. K., Abdel-Mohsen, D. M., Taha, H., Akabawy, A. M. A., Kassab, R. B., Abdel Moneim, A. E., & Amin, H. K. (2020). Neuromodulatory effects of green coffee bean extract against brain damage in male albino rats with experimentally induced diabetes. *Metabolic brain disease*, *35*(7), 1175–1187. https://doi.org/10.1007/s11011-020-00583-6

Allain, C. C. (1974). Cholesterol enzymatic colorimetric method. *J. of Clin. Chem*, 20, 470.

Alzheimer's Association; 2022. Alzheimer's Disease Facts & Figures Special Report; More Than Normal Aging: Understanding Mild Cognitive Impairment. 2022.

Aykac, A., Teralı, K., Ozbeyli, D., Ede, S., Albayrak, O., Başer, K. H. C., & Şener, G. (2022). A multi-parameter evaluation of the neuroprotective and cognitive-enhancing effects of Origanum onites L. (Turkish Oregano) essential oil on scopolamine-induced amnestic rats. *Metabolic brain disease*, *37*(4), 1041–1055.

Belfield, A., and Goldberg, D. M. (1971). Alkaline phosphatase colorimetric method. *J. of Enzyme*, (12): 561.

Bergmeyer, H. U., Scheibe, P. and Wahlefeld, A. W. (1978). Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clinical chemistry, 24(1), 58-73.

Breijyeh, Z. and Karaman, R., 2020. Comprehensive review on Alzheimer's disease: causes and treatment. Molecules 25.

Brinza I, Boiangiu RS, Hancianu M, Cioanca O, Erdogan Orhan I, Hritcu L (2021) Bay leaf (*Laurus Nobilis* L.) incense improved scopolamine-induced amnesic rats by restoring cholinergic dysfunction and brain antioxidant status. Antioxidants (Basel) 10(2):259.

Budryn G, Grzelczyk J, Jaśkiewicz A, Żyżelewicz D, Pérez-Sánchez H, Cerón-Carrasco JP. (2018). Evaluation of butyrylcholinesterase inhibitory activity by chlorogenic acids and coffee extracts assed in ITC and docking simulation models. Food Res Int 109:268-277.

Burns, J.M., Johnson, D. K., Watts, A., Swerdlow, R. H. and Brooks, W. M. (2010). Reduced lean mass in early Alzheimer disease and its association with brain atrophy, Arch. Neurol., , 67, 428–433.

Carneiro, S. M.; Oliveira, M. B. P. P.; Alves, R. C. (2021). Neuroprotective properties of coffee: An update. *Trends Food Sci. Technol.* , *113*, 167– 179.

Chapman, D.; R. Gastilla and J. Campbell (1959). Evaluation of protein in foods:1- A Method for the determination of protein efficiency ratio. *Can. J. Biochem. Phys* , 37: 679- 86.

Choi, B. K., Park, S. B., Lee, D. R., Lee, H. J., Jin, Y. Y., Yang, S. H. & Suh, J. W. (2016). Green coffee bean extract improves obesity by decreasing body fat in high-fat diet-induced obese mice. *Asian Pacific journal of tropical medicine*, *9*(7), 635–643.

Ciaramelli, C.; Palmioli, A.; Airoldi, C. (2019). Coffee variety, origin and extraction procedure: Implications for coffee beneficial effects on human health. *Food Chem.* , *278*, 47– 55.

Dhakal S, Kushairi N, Phan CW, Adhikari B, Sabaratnam V, Macreadie I. (2019). Dietary polyphenols: a multifactorial strategy to target Alzheimer's disease. Int J Mol Sci 20: 5090.

Draper, H. and Hadley, M. (1990). Malondialdehyde determination as index of lipid per-oxidation. *Methods Enzymol*,186: 421-431.

Dunant, Y. and V. Gisiger, V.(2017). Ultrafast and slow cholinergic transmission. Different involvement of acetylcholinesterase molecular forms, Molecules, 22, E1300.

El Rabey, H.A., Rezk, S.M., Sakran, M.I. *et al.*(2021). Green coffee methanolic extract and silymarin protect against CCl4-induced hepatotoxicity in albino male rats. *BMC Complement Med Ther* **21**, 19. https://doi.org/10.1186/s12906-020-03186-x.

Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology*, *7*(2), 88-95.

Elpasty, S., Helal, E., Mansoury, M., & Algendy, A. (2022). Impact of Green Coffee Extract on Body Weight and Physiological Indicators of Metabolic State in Obese Male Rats. *Egyptian Journal of Chemistry*, *65*(8), 715-723.

Farias-Pereira, R., Young, L. and Park, Y. (2021). Neuroprotective effects of green coffee bean extract against Alzheimer's and Parkinson's disease: a mini review. Food Life 2021(1):1-7.

Fassati, P. and Prencipe, L. (1982). Triglyceride enzymatic colorimetric method. *J. Clin. Chem*, 28, 2077.

Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*, *18*(6), 499-502.

Ghasemi, S., Moradzadeh, M., Hosseini, M., Beheshti, F. and Sadeghnia, HR. (2019) Benefcial effects of Urtica dioica on scopolamine-induced memory impairment in rats: protection against acetylcholinesterase activity and neuronal oxidative damage. *Drug Chem Toxicol* 42(2):167–175.

Hernández-Rodríguez, M., Arciniega-Martínez, I.M., García-Marín, I.D. *et al.* (2020). Chronic Administration of Scopolamine Increased GSK3βP9,

Beta Secretase, Amyloid Beta, and Oxidative Stress in the Hippocampus of Wistar Rats. *Mol Neurobiol* **57**, 3979–3988.

Hosseini, Z., Mansouritorghabeh, F., Kakhki, F. S. H., Hosseini, M., Rakhshandeh, H., Hosseini, A., Hasanpour, M., Iranshahi, M., & Rajabian, A. (2022). Effect of Sanguisorba minor on scopolamine-induced memory loss in rat: involvement of oxidative stress and acetylcholinesterase. *Metabolic brain disease*, *37*(2), 473–488.

Hussein, J., El-Khayat, Z., El-Toukhy, S., El-Bana, M., Medhat, D., & Morsy, S. (2016). Panax ginseng regulates brain monoamines in lipopolysaccharide-induced experimental brain injury. *Der Pharma Chemica*, *8*(6), 116-121.

Hussein, M.M.A., Samy, M., Arisha, A.H., Saadeldin, I.M., and Alshammari, G.M. (2020). "Anti-obesity effects of individual or combination treatment with Spirulina platensis and green coffee bean aqueous extracts in highf at diet-induced obese rats", Frontiers In Life Sci., 13 (1), pp. 328–338.

Ishida, K., Yamamoto, M., Misawa, K., Nishimura, H., Misawa, K., Ota, N., & Shimotoyodome, A. (2020). Coffee polyphenols prevent cognitive dysfunction and suppress amyloid β plaques in APP/PS2 transgenic mouse. *Neuroscience research*, *154*, 35–44.

Kanchanasurakit, S., Saokaew, S., Phisalprapa, P., & Duangjai, A. (2023). Chlorogenic acid in green bean coffee on body weight: a systematic review and meta-analysis of randomized controlled trials. *Systematic reviews*, *12*(1), 163.

Kaplan, L.A. (1984). Clin Chem. The C.V. Mosby co.st Louis. Toronto. Princeton; 1032-1036.

Kim J, Lee S, Shim J, Kim HW, Kim J, Jang YJ, Yang H, Park J, Choi SH, Yoon JH, Lee KW, Lee HJ (2012) Caffeinated coffee, decaffeinated coffee, and the phenolic phytochemical chlorogenic acid up-regulate NQO1 expression and prevent H(2)O(2)-induced apoptosis in primary cortical neurons. Neurochem Int 60(5):466–474.

Kim, J. H., , He, M. T., , Kim, M. J., , Yang, C. Y., , Shin, Y. S., , Yokozawa, T., , Park, C. H., , & Cho, E. J., (2019). Safflower (*Carthamus tinctorius* L.) seed attenuates memory impairment induced by scopolamine

in mice via regulation of cholinergic dysfunction and oxidative stress. *Food & function*, *10*(6), 3650–3659.

Konar A, Gupta R, Shukla K, Maloney B, Khanna V,Wadhwa R, Lahiri DK (2019). M1 muscarinic receptor is a key target of neuroprotection, neuroregeneration and memory recovery by i-Extract from *Withaniasomnifera*. Sci Rep 9:13990. https://doi.org/10.1038/s41598-019-48238-6.

Kumar, A., Sidhu, J., Goyal, A., Tsao, J.W., 2022. Alzheimer disease. StatPearls 1–27. Laferriere, C.A., Pang, D.S., 2020. Review of rodent euthanasia with pentobarbital. J. Am. Assoc. Lab Anim. Sci. 59, 254–263.

Lee TK, Kang IJ, Kim B, Sim HJ, Kim DW, Ahn JH, Lee JC, Ryoo S, Shin MC, Cho JH, Kim YM, Park JH, Choi SY, Won MH. (2020). Experimental pretreatment with chlorogenic acid prevents transient ischemia-induced cognitive decline and neuronal damage in the hippocampus through anti-oxidative and anti-inflammatory effects. Molecules 25:3578.

Li Q, He S, Chen Y, Feng F, Qu W, Sun H (2018). Donepezil-based multi-functional cholinesterase inhibitors for treatment of Alzheimer's disease. Eur J Med Chem 158:463–477.

Meng, S., Cao, J., Feng, Q., Peng, J. and Hu, Y. (2013). Roles of chlorogenic acid on regulating glucose and lipids metabolism: A review. *Evidence-Based Complementary and Alternative Medicine*, 801457. https://doi.org/10.1155/2013/801457.

Meng, S.X., Liu, Q., Tang, Y.J., Wang, W.J., Zheng, Q.S., Tian, H.J., Yao, D.S., Liu, L., Peng, J.H., Zhao, Y., Hu, Y., and Feng, Q. (2016). "A recipe composed of Chinese herbal active components regulates hepatic lipid metabolism of NAFLD in vivo and in vitro", BioMed Res Int., 2016,1026852.

Metwally DM, Alajmi RA, El-Khadragy MF, Yehia HM, AL-Megrin WA, Akabawy AMA, Amin HK, Abdel Moneim AE. (2020). Chlorogenic acid confers robust neuroprotection against arsenite toxicity in mice by reversing oxidative stress, inflammation, and apoptosis. J Funct Foods 75:104202.

Mohamed, H. E., Asker, M. E., Shaheen, M. A., Eissa, R. G., & Younis, N. N. (2021). Alleviation of fructose-induced Alzheimer's disease in rats by

pioglitazone and decaffeinated green coffee bean extract. *Journal of Food Biochemistry*, **45**(5), e13715.

Mohamed, S. M., Shalaby, M. A., Al-Mokaddem, A. K., El-Banna, A. H., El-Banna, H. A., & Nabil, G. (2023). Evaluation of anti-Alzheimer activity of Echinacea purpurea extracts in aluminum chloride-induced neurotoxicity in rat model. *Journal of Chemical Neuroanatomy*, *128*, 102234.

Molska, G. R., Paula-Freire, L. I. G., Sakalem, M. E., Köhn, D. O., Negri, G., Carlini, E. A., & Mendes, F. R. (2021). Green coffee extract attenuates Parkinson's-related behaviors in animal models. *Anais da Academia Brasileira de Ciencias*, *93*(suppl 4), e20210481. https://doi.org/10.1590/0001-3765202120210481

Mosallam, S. (2022). Micronucleus and Comet Assay as An Index for Carbon Tetrachloride Genotoxicity in Rats. *Egyptian Academic Journal of Biological Sciences. C, Physiology and Molecular Biology*, *14*(1), 325-336. doi: 10.21608/eajbsc.2022.231278.

Murray R. (1984). Clin Chem the C. V. mosby co. st Louis. Toronto. Princeton., 1088- 1090.

Owolabi J, Olatunji S, Olanrewaju A (2017). Caffeine and cannabis effects on vital neurotransmitters and enzymes in the brain tissue of juvenile experimental rats. Ann Neurosci 24(2):65–73.

Patton, C.J and Crouch S.R. (1977): Calorimetric determination of blood urea". Analyt. Chem., 49: 464-469.

Priftis A, Panagiotou E-M, Lakis K, Plika C, Halabalaki M, Ntasi G, Veskoukis AS, Stagos D, Skaltsounis LA, Kouretas D (2018) Roasted and green coffee extracts show antioxidant and cytotoxic activity in myoblast and endothelial cell lines in a cell specific manner. Food Chem Toxicol 114:119–127.

Reeves, P. Nielsen, F. and Fahmy, G. (1993). AIN-93. Purified diets for laboratory rodents: Final reports of the American Institute of Nutrition adhoe writing committee of reformulation of the AIN-76 A Rodent Diet. *J. Nutr.*, 123: 1939-1951.

Salamat, Sh.M., Haghighizadeh, MH., Haidari, F., Heli, B. (2018). The effect of green coffee bean extract supplementation on anthropometric indices, lipid profile and high-sensitivity c-reactive protein in adult men with dyslipidemia. J. Biochem. Technol. 2: 75-81.

Samoggia, A., & Riedel, B. (2019). Consumers' perceptions of coffee health benefits and motives for coffee consumption and purchasing. *Nutrients*, **11**(3), 653.

Snedecor, G.W. and Cochran, W.G. (1989). Statistical Methods. 8th Edition, Iowa State University Press, Ames.

Song SJ, Choi S, Park T (2014) Decaffeinated green coffee bean extract attenuates diet-induced obesity and insulin resistance in mice. Evidence-based complementary and alternative medicine 2014.

Spitz, D. R. and Oberley, L. W. (1989). An assay for superoxide dismutase activity in mammalian tissue homogenates. *Analytical biochemistry*, *179*(1), 8-18.

Tang KS (2019). The cellular and molecular processes associated with scopolamine-induced memory deficit: a model of Alzheimer's biomarkers. Life Sci 233:116695. https://doi.org/10.1016/j.lfs.2019.116695

Venkatakrishna, K., Sudeep, H. V., & Shyamprasad, K. (2021). Acute and sub-chronic toxicity evaluation of a standardized green coffee bean extract (CGA7™) in Wistar albino rats. SAGE open medicine, 9.

Xiaoyun He, Shujuan Zheng, Yao Sheng, Tong Miao, Jia Xu, Wentao Xu, et al. (2020). Chlorogenic acid ameliorates obesity by preventing energy balance shift in high-fat diet induced obese mice. J of science of the food and agriculture. https://onlinelibrary.wiley.com/doi/10.1002/jsfa.10675.

تأثير مستخلص حبوب القهوة الخضراء على ضعف الذاكرة المحدث بالسكوبولامين في الفئران

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الملخص العربي

هدفت هذه الدراسة إلى تقييم تأثير مستخلص حبوب البن الأخضر(GCBE) على ضعف الذاكرة المُستحث بالسكوبولامين لدى الفئران. تم تقسيم أربعون فأرًا إلى مجموعتين. المجموعة الأولى، ٨ فئران تغذت على النظام الغذائي الأساسي، وخُصصت كمجموعة ضابطة سلبية. المجموعة الثانية، ٣٢ فأرا (مجموعة السكوبولامين)، حُقِنت بالسكوبولامين (١ ملغ/جم مذاب في محلول ملحي) لمدة ٢٠ يومًا لتحفيز ضعف الذاكرة. بعد التأكد من تحفيز ضعف الذاكرة، قُسِّمت الفئران إلى أربع مجموعات فرعية: المجموعة الأولى، كمجموعة ضابطة إيجابية، وثلاث مجموعات فرعية مُعالجة، أُعطيت عن طريق الفم ١ مل/يوم من مستخلص البن الأخضر بتركيزات (٥٪، ٧.٥٪، و١٠٪) على التوالي. أظهرت النتائج أن السكوبولامين حفَّز نشاط الأستيل كولين إستراز، ومستويات بيتا أميلويد، والإجهاد التأكسدي. من ناحية أخرى، أظهر مستخلص حبوب البن الأخضر (GCBE) انخفاضًا ملحوظًا في نشاط أستيل كولين إستراز، ومستويات بيتا أميلويد، ومؤشرات الإجهاد التأكسدي (MDA)، مما يُحسّن الذاكرة ومستويات إنزيمات مضادات الأكسدة(SOD, CAT and GSH) لدى الفئران المُستحثة بالسكوبولامين. علاوة على ذلك، يُحسّن مستخلص حبوب البن الأخضر الوظائف العصبية (النورإبينفرين، والدوبامين، والسيروتونين)، ومستوى الدهون(TC,TG,LDL-C, HDL-C, VLDL-C) ، ووظائف الكبد (ALT,AST, ALP) والكلى (اليوريا والكرياتينين وحمض اليوريك) لدى الفئران. وبناءً على ذلك، تُبيّن هذه الدراسة الآثار الإيجابية لمستخلص حبوب البن الأخضر في علاج ضعف الذاكرة الناتج عن السكوبولامين. وختامًا، قد يُصبح مستخلص حبوب البن الأخضر عاملًا واعدًا لتحسين الذاكرة لدى مرضى الزهايمر والاضطرابات الشبيهة بمرض الزهايمر.

الكلمات المفتاحية: مستخلص ماء حبوب البن الأخضر، سكوبولامين، مرض الزهايمر، نشاط مضاد الكولينستريز، بيتا أميلويد، الإجهاد التأكسدي.